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Responses of *Heterodera glycines* and *Meloidogyne incognita* to exogenously applied neuromodulators

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Abstract

Biogenic amines regulate important behaviours in nematodes and are associated with pharyngeal activity in plant-parasitic nematodes. A robust behavioural assay based upon nematode body movements was developed to expand the study of these and other neuroregulators in plant-parasitic nematodes. Dopamine, octopamine and serotonin each had significant but differing effects on the behaviour of soybean cyst nematode *Heterodera glycines* and root-knot nematode *Meloidogyne incognita* juveniles. Body movement frequency was increased twofold in *H. glycines* by 5 mM dopamine ($P = 0.0001$), but decreased by 50 mM dopamine in *H. glycines* (88%) and *M. incognita* (53%) ($P < 0.0001$). Movement frequency in both species was increased by 50–70% ($P < 0.0001$) by 50 mM octopamine, and 5 mM octopamine increased *M. incognita* movement frequency more than twofold ($P < 0.0001$). Movement frequency in each species was reduced by more than 90% by 5 mM serotonin ($P < 0.0001$). While amplitude of body movement in *H. glycines* was unaffected by any amine, it was significantly reduced in *M. incognita* by all amines ($P < 0.0006$). Stylet pulsing frequencies in either species were unaffected by dopamine or octopamine, but 5 mM serotonin stimulated pulsing in *H. glycines* by nearly 13-fold ($P < 0.0001$) and in *M. incognita* by more than 14-fold ($P < 0.0001$). The invertebrate neuropeptide FLRFamide (*N*-Phe-Leu-Arg-Phe) increased *M. incognita* body movement frequency 45% ($P = 0.02$) at 1 mM but did not affect stylet activity. Finally, *H. glycines* egg hatch was completely suppressed by 50 mM serotonin, and partially suppressed by 50 mM dopamine (75%; $P < 0.0001$) and 50 mM octopamine (55%; $P < 0.0001$).

Introduction

Nematodes rely upon biogenic amines (BA) and neuropeptides to regulate essentially all aspects of development and physiology (Komuniecki *et al.*, 2004; Perry & Maule, 2004). Research on the biochemistry, pharmacology and genetics of BAs and their receptors in animal-parasitic nematodes, primarily *Ascaris suum*, and the free-living nematode *Caenorhabditis elegans*, is extensive (Horvitz *et al.*, 1982; Chaudhuri & Donahue, 1989; Williams *et al.*, 1992; Trim *et al.*, 2001; Komuniecki *et al.*, 2004; Perry & Maule, 2004; Hobson *et al.*, 2006). Such studies have been driven by veterinary and medical interests, and by the status of *C. elegans* as a model organism. Somewhat less effort has been devoted to analysis of BAs and their effects in plant-parasitic nematodes that are of particular importance to agriculture. Serotonin and octopamine were found to have varying effects on behaviour in the plant-parasitic nematodes *Heterodera schachtii* and *Meloidogyne incognita*

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(McClure & von Mende, 1987), and the involvement of serotonin in *H. schachtii* reproduction has been examined (Jonz *et al.*, 2001). Octopamine and serotonin have been used to induce uptake of double-stranded RNA (dsRNA) by plant-parasitic nematodes to facilitate gene silencing studies (Urwin *et al.*, 2002; Bakhetia *et al.*, 2005; Chen *et al.*, 2005; Rosso *et al.*, 2005).

The present work examines effects of the BAs dopamine, octopamine and serotonin on specific behaviours of juveniles (J_2) of two different genera of plant-parasitic nematodes, *H. glycines* and *M. incognita*. The same amines were also examined for their effects on egg hatch in *H. glycines*. Finally, the response of *M. incognita* juveniles to the invertebrate neuropeptide FLRFamide (*N*-Phe-Leu-Arg-Phe), a member of a neuropeptide family known to be involved with neuromuscular regulation in nematodes (Mousley *et al.*, 2004; Li, 2005), was also assessed. The purpose of the work was to compare the responses of different plant-parasitic nematodes to the same biogenic amine treatments, and to provide a robust behavioural assay that could be used to examine the effects of other neuroregulators such as neuropeptides. Presented here are the effects of dopamine, octopamine, serotonin and the neuropeptide FLRFamide on behaviours associated with body wall and pharyngeal muscular activities.

Materials and methods

Nematodes

Heterodera glycines was raised on soybean (*Glycine max*, cv. Kent) grown in sand-filled beakers using a constant moisture system (Sardanelli & Kenworthy, 1997), at 27°C and 16 h light:8 h dark photoperiod. Cultures were inoculated with 2000 *H. glycines* eggs/plant. Approximately 5 weeks after inoculation, plants were harvested and females and eggs were collected from roots. Eggs were hatched on a modified Baerman funnel, and hatched J_2 were collected daily for use in assays. *Meloidogyne incognita* was raised on pepper (*Capsicum annuum*, cv. Yolo Wonder) grown in the greenhouse at 24–29°C and a 16 h light:8 h dark photoperiod. Plants were harvested after 3 months and eggs collected. Eggs were hatched in water in glass dishes fitted with nylon mesh (30 μm pore size) that retained eggs but allowed freshly hatched J_2 to pass through and into the bottom of the dish. Juveniles were collected fresh daily and used immediately.

Reagents

Dopamine, octopamine, serotonin (5-hydroxytryptamine), and the amidated neuropeptide N-Phe-Leu-Arg-Phe (FLRFamide) were from Sigma (St. Louis, Missouri, USA). Each dissolved readily in water, and stock solutions were prepared fresh daily for each experiment.

Treatments and measurements

Freshly collected J_2 were transferred into 1.5 ml polypropylene tubes, containing either tap water (controls) or test solutions prepared in tap water, at a density of $\sim$1 J_2/1.5–2 μl of solution. Total volumes were 200–400 μl/tube, and J_2 were incubated at room temperature for at least 10 min prior to aliquot collection. To monitor J_2 activity and behaviour, aliquots (15 μl drops) containing $\sim$7–10 J_2 were placed on a glass microscope slide, and observed at either 40 × or 100 × magnification.

Observations were made separately for body movement and stylet activity. Body movement was monitored for both frequency and amplitude of lateral motion of the anterior portion of the worm. Lateral motion was defined as any change in direction from a previous position, and could occur in any plane. Using the lengthwise axis of the worm as a reference, each movement was scored for degree of motion. Locomotion was not scored. Each complete motion was counted as one movement, and each animal was observed for at least 1 min. Data for each animal were recorded as movements/min (frequency), average degrees/min (amplitude), and total degrees of motion/min. Stylet activity typically was a mild thrusting and retraction (pulsing) of the stylet. Up to $\sim$60 pulses/min could be counted, and this was considered low to moderate activity. More than 60 pulses/min was considered fast. Stylet activity was quantified by using category scoring rather than by absolute stylet movement count, since we found a lack of accuracy in measuring stylet movement rates of more than 60/min. Stylet activity for each J_2 was scored as 0 (no movement), 1 (low to moderate movement), and 2 (fast movement), and each J_2 was observed for at least 1 min.

For frequency experiments, the length of exposure of individual worms varied from 10 to 30 min or 60–80 min, and were referred to as 10-min and 60-min assays. For amplitude and stylet experiments, exposure times were typically between 10 and 50 min. In addition to the biogenic amine assays in *M. incognita*, body movement and stylus activity assays were also done using FLRFamide, a member of a large family of invertebrate neuropeptides, the FMRFamide-like peptides (FLPs). FLPs are known to have behavioural effects in nematodes (Li, 2005), and the C-terminal FLRFamide motif has been identified in a number of predicted FLP homologues in *M. incognita* (McVeigh *et al.*, 2005).

Egg hatch assays were conducted at 27°C in 24-well polystyrene plates (Corning, Corning, New York, USA). Each well contained 200–250 *H. glycines* eggs in a total of 400 μl of tap water or biogenic amine treatment solution. Immediately after loading the wells, all eggs and J_2 were counted to provide accurate starting (baseline) values. Daily thereafter, J_2 in all wells were counted and net J_2 calculated by subtracting the baseline J_2 value for each well. The ratio of net J_2 to starting egg value was used to calculate cumulative percent hatch. All treatments were replicated at least three times.

Data analysis

Data for frequency, amplitude, total degree or stylet scores are expressed as the means ± standard error mean (SEM) and are compared using Student's *t*-test and one-way analysis of variance (ANOVA). Hatch data are expressed as the means ± standard error mean (SEM) and are compared using one-way ANOVA. All data were analysed statistically using KaleidaGraph (Synergy Software, Reading, Pennsylvania, USA).

Results

Heterodera glycines

Frequency and amplitude of movement

The BAs tested each had significant and differing effects upon *H. glycines* J_2 behaviour, as measured by the frequency of body movements (table 1). At 5 mM, neither dopamine nor octopamine had any effect when assayed at 10 min, but dopamine nearly doubled the frequency of movement over the control at 60 min ($P = 0.0001$), whereas octopamine had only a marginal stimulatory effect ($P = 0.061$) compared to the control. In contrast, 5 mM serotonin drastically depressed activity at 10 min ($P < 0.0001$) and completely eliminated activity at 60 min. Serotonin at 1 mM was also effective at reducing activity at 10 min (50% reduction, $P = 0.0037$), and 0.5 mM serotonin marginally reduced activity at 60 min ($P = 0.060$) but had no effect at 10 min. Increasing the dopamine and octopamine doses to 50 mM had opposite effects. Dopamine depressed activity by 88% at 10 min ($P < 0.0001$), but was less effective over time (48% reduction at 60 min, $P = 0.06$), while 50 mM octopamine increased activity, by 49% at 10 min ($P < 0.0001$) and 46% at 60 min ($P = 0.0002$).

Separate experiments were conducted to examine effects of selected doses of BAs on amplitude of movement. Biogenic amines were selected on the basis of their effects during frequency of movement assays. Dopamine at 5 mM (no effect to stimulatory), 50 mM octopamine (stimulatory), and 0.5 mM serotonin (no effect to inhibitory) were assayed (table 2). Frequencies of movements were as expected for 10-min assays (table 1), with no effects by 5 mM dopamine or 0.5 mM serotonin, and a frequency of movement increase by 50 mM octopamine (40%, $P < 0.0001$). None of the treatments had any effect upon amplitude of movements (table 2). Thus the 22% increase in mean total degrees/min by the 50 mM octopamine treatment ($P = 0.065$) was dependent upon frequency of movement, and the mean total degrees/min of the other treatments did not vary from that of the control.

Table 1. Effect of biogenic amines on *Heterodera glycines* juvenile behaviour*.

Treatment group†	10 min	60 min
Group 1: dopamine		
control	9.60 ± 1.18[a]	9.20 ± 1.26[a]
5 mM	11.20 ± 1.96[a]	18.30 ± 1.38[b]
50 mM	1.20 ± 0.55[c]	4.80 ± 2.18[c]
Group 2: octopamine		
control	10.02 ± 0.48[a]	11.00 ± 0.70[a]
5 mM	10.94 ± 0.99[a]	13.17 ± 0.87[c]
50 mM	14.89 ± 0.56[b,c]	15.11 ± 0.71[b]
Group 3: serotonin		
control	10.48 ± 0.58[a]	11.00 ± 0.89[a]
0.5 mM	11.41 ± 0.84[a]	6.78 ± 1.83[d]
1 mM	5.25 ± 1.05[b]	0 ± 0[e]
5 mM	0.18 ± 0.18[c]	0 ± 0[e]

*Data are expressed as the mean ± SEM of head movements/min (frequency) for at least ten different animals within each treatment group (except serotonin 0.5 mM, 60 min, where $N = 9$). The same treatment groups were used for 10-min and 60-min observations.

†Means are compared within each treatment group using Student's *t*-test. Means followed by the same letter are not significantly different. In addition, all control means were compared by one-way ANOVA with no differences found ($P = 0.70$).

Stylet behaviour

Stylet activity was examined with the same BAs and concentrations used in the body movement frequency assays (see table 1). Neither dopamine nor octopamine treatments had any effect on stylet activity (data not shown). However, serotonin at 1 mM and 5 mM stimulated stylet pulsing over the control. The mean stylet pulse score/J_2 for 1 mM serotonin (0.83 ± 0.22) was a 5.9-fold increase over the control (0.014 ± 0.07; $P = 0.007$), and 5 mM serotonin caused a 12.7-fold increase (1.78 ± 0.09; $P < 0.0001$).

Egg hatch

Egg hatch was unaffected by either 5 mM dopamine or 5 mM octopamine (fig. 1), but each depressed hatching at 50 mM. Mean percent egg hatch on day 5 with 50 mM dopamine (15.08 ± 2.06) fell 75% ($P < 0.0001$) from the control value (60.98 ± 2.08), and mean percent hatch in 50 mM octopamine (27.32 ± 2.15) was reduced by 55% ($P < 0.0001$) from the control. Serotonin reduced egg hatch by 88% at 5 mM (7.06 ± 0.74; $P < 0.0001$), and eliminated hatch at 50 mM (fig. 1). The response of *H. glycines* egg hatch to serotonin exposure was dose-dependent (fig. 2). On day 2 of exposure to 0.5 mM serotonin, the mean cumulative percent hatch (15.78 ± 1.13) was 50% less than controls (33.97 ± 1.80; $P = 0.0018$), and with a 2 mM exposure the hatch rate was reduced more than 90% ($P = 0.001$). Depression of egg hatch after 5 days' exposure was somewhat less severe, expressed as a percentage of the control, than at 2 days. Nonetheless, egg hatch was still significantly reduced, with mean cumulative percent hatch down 26% with 0.5 mM ($P = 0.0003$), 78% at 2 mM ($P < 0.0001$) and almost 90% at 5 mM ($P < 0.0001$).

Meloidogyne incognita

Frequency and amplitude of movement

Frequency of motion in *M. incognita* was affected by BA and neuropeptide treatments (fig. 3). Mean frequencies of movement for 50 mM dopamine (6.93 ± 1.60) and 5 mM serotonin (1.35 ± 0.51) were 53% and 91% lower, respectively, than the control level (14.62 ± 0.58; $P < 0.0001$). Octopamine caused increases in frequency of movement at 50 mM (25.10 ± 10.93; $P < 0.0001$) and 5 mM (30.85 ± 1.68; $P < 0.0001$) to between 70 and 110%. The results with BAs are qualitatively similar to those obtained with *H. glycines* (table 1). *Meloidogyne incognita* also responded to the neuropeptide FLRFamide by an increase in movement frequency of 45% (21.20 ± 2.33; $P = 0.02$) with a 1 mM dose (fig. 3).

Three treatments (5 mM and 50 mM dopamine and 50 mM octopamine) were examined for their effects on amplitude of movement (table 3), and all three resulted in

Table 2. Effect of biogenic amines on amplitude of *Heterodera glycines* juvenile anterior movement*.

Treatment	Frequency†	Amplitude†	Degrees/min†
Control	10.39 ± 0.58[a]	122.16 ± 6.75[a]	1317.41 ± 99.24[a]
5 mM dopamine	9.83 ± 0.90[a]	125.82 ± 14.31[a]	1283.89 ± 164.81[a]
50 mM octopamine	14.60 ± 0.63[b]	108.80 ± 5.34[a]	1610.50 ± 120.54[b]
0.5 mM serotonin	9.71 ± 1.05[a]	130.09 ± 10.66[a]	1337.35 ± 185.11[a]

*Data are expressed as the mean ± SEM of number of head movements/min (frequency), degrees covered/head movement (amplitude), and total degrees covered per individual J_2/min for at least 17 different animals/treatment.
†Means are compared across treatments using Student's *t*-test, and values within a column followed by the same letter are not significantly different ($P > 0.10$).

significantly reduced amplitude. With 5 mM dopamine, amplitude was 20% lower than controls ($P = 0.0006$). Decreases of 55% ($P < 0.0001$) and 40% ($P < 0.0001$) were observed with 50 mM dopamine and octopamine, respectively. The corresponding reductions in total degrees/min for dopamine treatments can be attributed to amplitude loss (table 3), and lack of change in degrees/min for 50 mM octopamine treatments relative to the control is a result of increased frequency and decreased amplitude of movement. These results contrast with those in *H. glycines*, where amplitude was not affected by any treatment (table 2).

Stylet behaviour

Stylet activity in *M. incognita* was unaffected by either 5 mM dopamine or 5 mM octopamine (data not shown), but stylet pulsing was increased 14-fold ($P < 0.0001$) after exposure to 5 mM serotonin, with a mean stylet score of 0.78 ± 0.10 compared to 0.05 ± 0.04 for the control. FLRFamide had no effect on stylet activity in *M. incognita* at either 1 mM or 0.2 mM ($N = 10$ each dose).

Discussion

Heterodera glycines and *M. incognita* J_2 responded to serotonin, dopamine and octopamine with a variety of changes in body activity and stylet motion. In addition, *H. glycines* egg hatch was reduced by serotonin, and *M. incognita* body movement frequency was increased by a neuropeptide. Dopamine, octopamine and serotonin are known to be involved in the regulation of numerous processes in nematodes essential for reproduction and survival, including egg laying, locomotion, muscular contraction and pharyngeal pumping, and response to the environment (Sawin *et al.*, 2000; Jonz *et al.*, 2001; Niacaris & Avery, 2003; Komuniecki *et al.*, 2004; Perry & Maule, 2004).

Biogenic amine modulation of neuronal and muscular activity is biochemically and physiologically complex, making precise descriptions of cause and effect difficult. For example, receptors for some nematode BAs have been detected and characterized, primarily from *A. suum* and *C. elegans* (Chaudhuri & Donahue, 1989; Williams *et al.*, 1992; Trim *et al.*, 2001; Rex & Komuniecki, 2002;

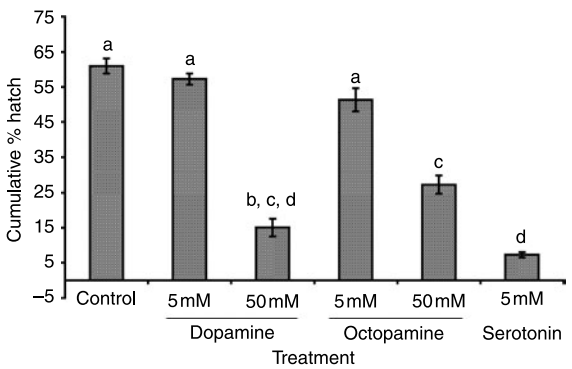

Fig. 1. Effect of biogenic amines on egg hatch in *Heterodera glycines*. Eggs from *H. glycines* were incubated in wells of 24-well polystyrene plates as described. Wells contained ~200–250 eggs in 400 μl of the indicated solution. Eggs and juveniles were counted in each well, juveniles were counted after 5 days, and net juveniles used to calculate percent hatch. Data are expressed as mean ± SEM of at least six separate observations, except for 50 mM treatments ($N = 3$). Means were compared using one-way ANOVA and means followed by the same letter are not significantly different ($P > 0.01$).

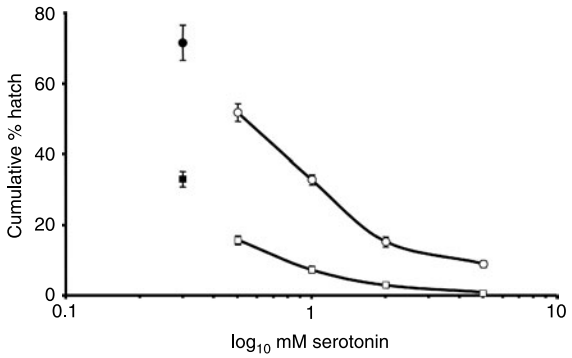

Fig. 2. Dose effects of serotonin on egg hatch in *Heterodera glycines*. Eggs from *H. glycines* were incubated in wells of 24-well polystyrene plates as described. Wells contained ~200–250 eggs in 400 μl of serotonin dilutions. Eggs and juveniles were counted in each well, juveniles were counted after 2 and 5 days, and net juveniles used to calculate percent hatch. Data are expressed as mean ± SEM of at least three separate observations for each data point. Day 2 control, filled square; Day 2 treatments, open squares; Day 5 control, filled circle; Day 5 treatments, open circles.

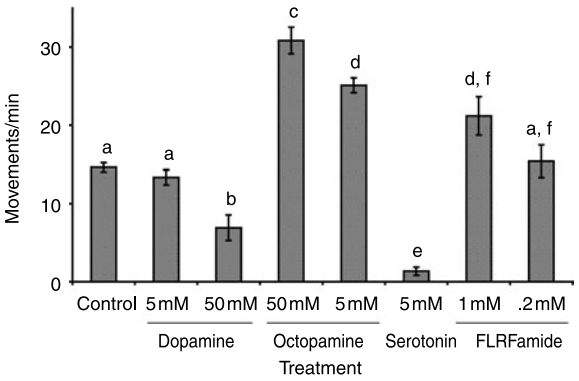

Fig. 3. Biogenic amine and neuropeptide effects on frequency of movement in juveniles of *Meloidogyne incognita*. Data are expressed as the mean ± SEM of head movements/min (frequency) for at least 20 different animals (except FLRFamide, where $N = 10$). Means are compared by one-way ANOVA and those followed by the same letter are not significantly different ($P > 0.01$).

Suo *et al.*, 2004; Sugiura *et al.*, 2005; Hobson *et al.*, 2006). Serotonergic receptors are relatively well characterized, but their expression is complex, and numerous variants can be present within an organism (Komuniecki *et al.*, 2004). Multiple isoforms of a dopamine receptor have been characterized in *C. elegans* (Suo *et al.*, 2003; Sanyal *et al.*, 2004), exhibiting variable binding affinities and, like serotonin receptors, are expressed in a variety of muscular and neuronal locations (Tsalik *et al.*, 2003; Komuniecki *et al.*, 2004). Receptors for octopamine have not been characterized, but this BA does bind to receptors associated with other BAs, including dopamine (Sanyal *et al.*, 2004) and tyramine (Rex & Komuniecki, 2002). Despite an incomplete understanding of its mode of action, octopamine is known to function as a neuromodulator, as do other BAs (Horvitz *et al.*, 1982; Sawin *et al.*, 2000), and contributes to the plasticity and complexity of nematode behaviour.

Serotonin (~20 mM) was reported to stimulate body movement and stylet activity in *M. incognita*, and ~20 mM octopamine stimulated body movement but not stylet activity (McClure & von Mende, 1987). However, these observations were done after 18 h and the responses were not characterized. We observed rapid responses to BAs in *H. glycines* and *M. incognita*, and detected clear qualitative and quantitative differences in the responses of these two nematodes. Decreased body movement in *H. glycines* exposed to 5 mM serotonin correlated with decreased locomotion reported in *C. elegans* (Horvitz *et al.*, 1982). Octopamine reduced locomotion in *C. elegans* (Horvitz *et al.*, 1982) but increased body movement in both *H. glycines* and *M. incognita* in this study.

Until now, dopamine has not been closely examined in plant-parasitic nematodes, but results with *H. glycines* body movement suggest more functional similarity with serotonin than with octopamine; but in stylet activity assays (see below), dopamine was similar to octopamine. Dopamine is similar to both octopamine and serotonin, as it elicits effects on *H. glycines* and *M. incognita* J_2 quite rapidly.

Although stylet pulsing and pharyngeal pumping are not equivalent and involve different musculatures, they may be responsive to similar receptors binding the same ligands. The stimulation by serotonin of pharyngeal pumping in *C. elegans* (Horvitz *et al.*, 1982) and the serotonin-stimulated stylet pulsing observed in *H. schachtii* (Jonz *et al.*, 2001) are responses similar to the enhanced stylet pulsing in *H. glycines* and *M. incognita*. Of note here is the increase in amphidial exudate that accompanies the increased stylet activity in *M. incognita* exposed to serotonin (McClure & von Mende, 1987).

Dopamine and octopamine each failed to stimulate stylet pulsing in our experiments with *H. glycines* and *M. incognita*. Similarly, octopamine failed to affect stylet activity in *M. incognita* after long-term exposure to octopamine (McClure & von Mende, 1987). Also, pharyngeal pumping in *C. elegans* was depressed by octopamine (Horvitz *et al.*, 1982). These results with octopamine are curious since it has been reported to increase the uptake of dsRNA by *H. glycines* and *Globodera pallida*, presumably by affecting pharyngeal pumping (Urwin *et al.*, 2002), and has been used in *G. rostochiensis* and *M. incognita* for the same purpose (Bakhetia *et al.*, 2005; Chen *et al.*, 2005). Also, Rosso *et al.* (2005) used uptake of the fluorescent dye fluorescein isothiocyanate (FITC) by *M. incognita* to assess levels of pharyngeal pumping in response to serotonin and octopamine. No uptake was observed after 1 h of exposure to either BA, but after exposure to 50 mM serotonin for 24 h approximately 80% of treated *M. incognita* had taken up FITC.

Table 3. Effect of biogenic amines on amplitude of *Meloidogyne incognita* juvenile anterior movement*.

Treatment	Frequency†	Amplitude†	Degrees/min†
Control	13.19 ± 0.54[a]	117.28 ± 4.30[a]	1518.50 ± 75.29[a]
5 mM dopamine	12.17 ± 0.80[a]	93.49 ± 5.03[b]	1155.00 ± 94.97[b]
50 mM dopamine	5.05 ± 0.84[b]	53.17 ± 3.58[c]	279.47 ± 55.98[c]
50 mM octopamine	25.10 ± 0.93[c]	70.31 ± 3.41[d]	1734.00 ± 84.53[a]

*Data are expressed as the mean ± SEM of number of head movements/min (frequency), degrees covered/head movement (amplitude), and total degrees covered per individual J_2/min for at least 17 different animals/treatment.
†Means are compared across treatments using Student's *t*-test and values within columns followed by the same letter are not significantly different ($P > 0.10$).

Less than 10% of the nematodes exposed to 50 mM octopamine showed any signs of uptake. The nature of pharyngeal pumping, stylet pulsing and body wall muscle activity responses in different species must vary not only with regard to the BA used, but to dosage and time as well.

A striking difference existed between *H. glycines* and *M. incognita* in the effect of BAs on the amplitude of body movement. While each species exhibited some response to BA treatment in the form of increased or decreased body movement frequency, only *M. incognita* responded by changing the amplitude of each body movement. Amplitude was reduced, as shorter movements were taken relative to the control, by dopamine, octopamine and serotonin. Further, the effects on amplitude appeared to be independent of frequency, since amplitude was reduced whether frequency increased, decreased or remained unchanged. This implies a high degree of complexity in the regulation of neuromuscular activities in plant-parasitic nematodes.

The rapid response of nematodes to treatment by BAs suggested that these neuromodulators may bind to cellular receptors in tissue very near the body wall, or may diffuse rapidly across the cuticle to access receptors on interior membranes. Knowledge of the biochemistry and pharmacology of BA receptors in nematodes is still developing (Trim *et al.*, 2001; Rex & Komuniecki, 2002; Komuniecki *et al.*, 2001; Rex *et al.*, 2004; Sanyal *et al.*, 2004; Suo *et al.*, 2004), but whatever the molecular mechanism, the response to ligands is very rapid.

The differing responses of two behaviours, stylet pulsing and body movement, in both *H. glycines* and *M. incognita* exposed to serotonin, are in accordance with observations made in other nematodes. Trim *et al.* (2001) reported that serotonin stimulates pharyngeal pumping in *A. suum* while reducing body wall muscle contractions, and *A. suum* locomotion is inhibited by serotonin (Reinitz & Stretton, 1996). The modulation by serotonin of pharyngeal pumping and locomotion in *C. elegans* (Horvitz *et al.*, 1982; Avery & Horvitz, 1990; Sawin *et al.*, 2000; Hardaker *et al.*, 2001; Niacaris & Avery, 2003) is coupled to nutritional state, and animals encountering food undergo a serotonin-mediated slowing response coupled to increased pharyngeal pumping (Sawin *et al.*, 2000; Niacaris & Avery, 2003).

In *C. elegans*, the *flp*-1 gene encodes a number of FLPs containing the C-terminal FLRFamide motif, some of which are associated with pharyngeal pumping and locomotion, along with other behaviours (Rogers *et al.*, 2001; Li, 2005). Homologues of these peptides are predicted to be expressed in a number of parasitic nematodes including *M. incognita* (McVeigh *et al.*, 2005). Given the involvement of the FLPs in *C. elegans* physiological and behavioural regulation (Li, 2005), and evidence for FLP receptors on *A. suum* body wall muscle (Mousley *et al.*, 2004; Rex *et al.*, 2004), it is intriguing that FLRFamide caused a rapid increase in the frequency of *M. incognita* body movement. It is possible that peptidergic receptors in *M. incognita* rapidly recognize this FLP. It is also interesting that FLRFamide had no observable effect on *M. incognita* stylet activity, placing the peptide in the same league as octopamine relative to behavioural effects on *M. incognita*. Additional analyses, including co-treatment of worms with the two agents, use of dose–response assays and screening of FLRFamide homologues, are needed to reveal the relationship between octopamine and FLPs in affecting behaviour.

The ability of BAs to penetrate the eggshell was confirmed by the serotonin inhibition of egg hatch in *H. glycines*. How rapidly serotonin enters the egg is not known, but hatching effects were significant within 2 days of treatment. Although still significant at 5 days, the percent hatch inhibition was reduced slightly, suggesting decreased serotonin potency, allowing partial recovery of the affected pre-hatch juveniles. In fact, a preliminary experiment with 5 mM serotonin indicated that *H. glycines* juveniles return to normal body and stylet activity overnight after transfer to control conditions. Another explanation of apparent recovery of hatching ability over time is stage of juvenile development. Juveniles in earlier and later developmental stages may respond differently to the same chemical, and as development proceeds an increased percentage of juveniles can initiate hatch. How serotonin affects egg hatch is unknown. Increases in frequency of head movement and in stylet pulsing would suggest that rupturing of the eggshell by pre-hatch juvenile activity should increase. However, decreased hatch suggests that the additional activity may not be coordinated normally, and that the energy spent is energy wasted.

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